REGULATION OF GENE EXPRESSION IN ENDOTHELIAL CELLS EXPOSED TO FLOW AND MECHANICAL STRAIN - Larry V. McIntire, Department of Chemical Engineering, Institute of Biosciences and Bioengineering, Rice University, Houston, TX, USA 77251-1892

Fluid shear stress can stimulate secretion of tissue plasminogen activator (tPA) by cultured human endothelial cells, while plasminogen activator inhibitor type-1 secretion remains unstimulated. To determine whether hemodynamically-induced changes in tPA messenger RNA (mRNA) levels also occur, cells from the same primary culture of human umbilical vein endothelial cells (HUVEC) were maintained in stationary culture or exposed to arterial levels of shear stress (25 dynes/cm2) for 24 hours. Total cellular RNA was isolated from the shear stressed and stationary cultures and the relative levels of tPA mRNA and glyceraldehyde 3-phosphate dehydrogenase (GAPDH) mRNA were determined using a coupled reverse transcriptase/polymerase chain reaction (PCR) method. As indicated by the amount of amplification product, tPA-mRNA levels were many fold higher (>10) in endothelial cells subjected to shear stress for 24 hours than in stationary controls. In contrast, mRNA level for GAPDH were similar in control and shear stressed cells. The constancy of the measured GAPDH signal indicated that the tPA response was a selective effect of fluid shear stress. Using the PCR method, the mRNA levels of basic fibroblast growth factor (bFGF) were found not to vary in comparison to GAPDH mRNA after 24 hours of shear stress. HUVEC secretion of endothelin, a potent vasoconstrictor and smooth muscle cell mitogen, was down regulated (by 90%) after 2 to 4 hours of exposure to shear stress. The level of endothelin mRNA was almost undetectable in cells exposed to shear stress for 24 hours. To examine the signal transduction mechanism involved in stress modulation of intracellular metabolism, measurements of inositol trisphosphate (IP_3) and intracellular free calcium were made using a radio immunoassay and fluorescence videomicroscopy respectively. Striking transient increases in IP_3 were measured in the time frame of 0-5 minutes after initiation of arterial stress. Calcium changes appeared to be dependent on ATP in the medium and therefore represent an effect of mass transfer. These results indicate that some of the enhancement of the fibrinolytic potential of endothelial cells in response to hemodynamic forces could involve transduction of shear stress to initiate transcriptional events. Also, the thickening of the vessel wall in low shear stress zones near arterial bifurcations could be due to over expression of endothelin. Intimal thickening is not found in high stress zones and this absence may be due to reduced production of endothelin by the shear stressed endothelium.

The effects of cyclical strain were modeled by culturing bovine aortic endothelial cells on fibronectin-coated elastic membranes of silicone rubber (Silastic®) or polyetherurethane urea (Mitrathane™). After growing to confluence under static conditions of 37°C in humidified air with 5% $C0_2$, cells were strained cyclically at membrane elongations of 5% or 10% for 24 hours at 1 Hz. Controls were maintained under static conditions or were exposed to fluid motions similar to the strained cells but without stretching. Secretion rates were constant throughout experiments in the strain chamber with no initial burst in metabolism associated with the initiation of strain. Secretion rates were not altered by choice of elastic membrane. At a physiological level of 10% cyclical strain, prostacyclin and endothelin secretion rates were increased by 2.5 fold and 1.7 fold, respectively, above stationary controls. Endothelin production demonstrated a dose dependent response with cyclical strain while PGI_2 appeared to require a threshold strain before an increase in secretion occurred. No significant differences in t-PA levels were seen in cyclically strained cells compared to controls. These results indicate that endothelial cells respond metabolically to cyclical strain and suggest that mechanical strain may modulate secretion of selective vasoactive materials by vascular endothelial cells.

Source: https://www.industrydocuments.ucsf.edu/docs/nqnv0000